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Haffkine Institute, P. J. DEORAS.
Bobmay, *February* 27, 1967. KARUNA KARNIKAR.

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ISOLATION OF *NOCARDIA BRASILIENSIS* FROM SOIL

IN the course of studies on the natural habitats of human pathogenic fungi undertaken in this laboratory, *Nocardia brasiliensis*, one of the important etiologic agents of mycetoma was encountered in one of the soil samples examined from Gwalior. This finding is worth reporting in view of the fact that this pathogen has been infrequently reported from soil.[1-3] From India *N. brasiliensis* has been reported from cases of mycetoma in man[4] but apparently there is no record so far of its isolation from soil.

Samples of soil were collected in sterile 1 oz. screw-capped bottles from various sites (depth not exceeding 5 cm.) and studied by the paraffin bait technique. The details of the methods used in the isolation and identification of *Nocardia* species were the same as given in a previous paper.[5] Pathogenicity of the strain isolated was tested in white mice. Growth from 2 weeks old culture on 4 slants of Sabouraud's glucose agar incubated at 37° C. was scraped and ground to a fine suspension with 5 ml. of sterile normal saline. An equal amount of 5% sterile hog gastric mucin was added to the suspension. Twelve male mice were divided into three equal groups of four animals. They were inoculated intraperitoneally with 0·25 ml. in one group, with 0·5 ml in the second group and with 1 ml. in the third group.

The solitary isolate of *N. brasiliensis* originated from one of the samples of garden soil collected in June 1966 from Gwalior, Madhya Pradesh. A brief account of the morphological and physiological characteristics and of pathogenicity of this isolate for laboratory animals is given below :

Colonies on Sabouraud's glucose agar (Fig. 1) were raised, folded and pale yellow, without

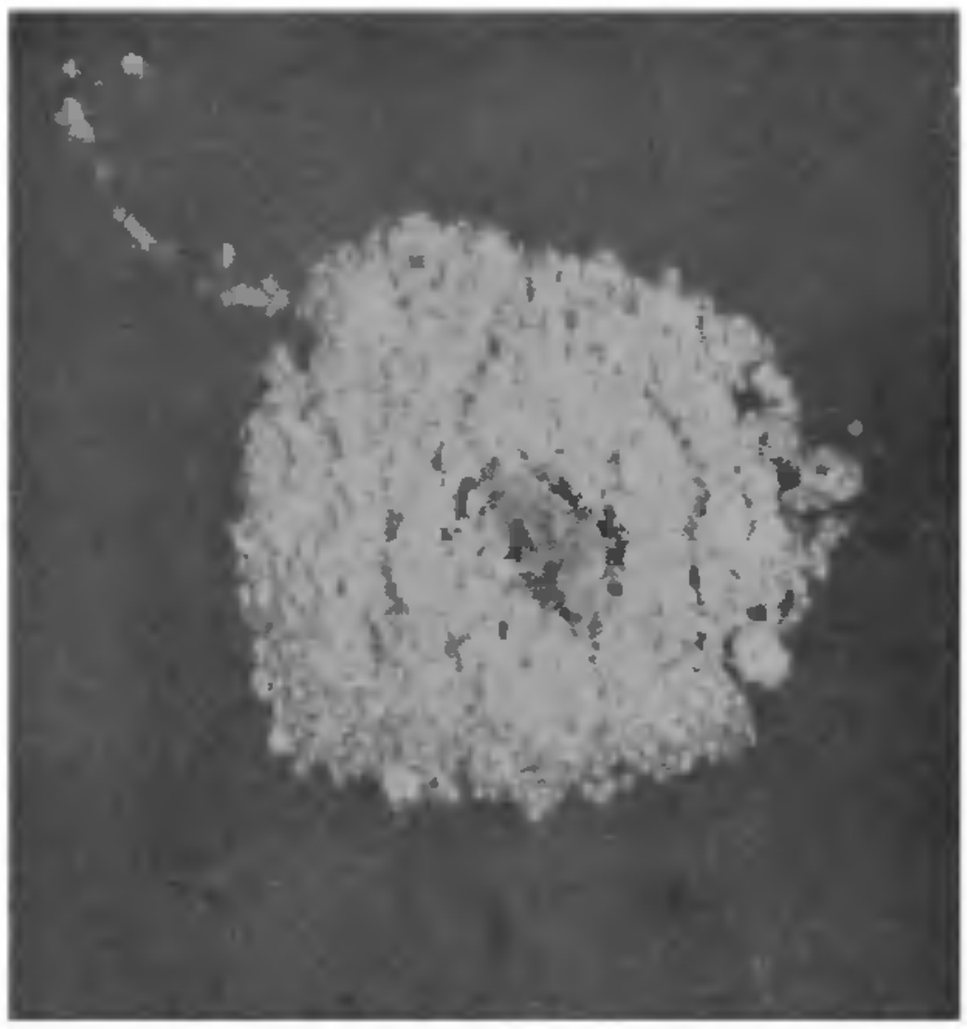

FIG. 1. Two weeks old colony of *Nocardia brasiliensis* on Sabouraud's glucose agar incubated at 37° C.

any diffusible pigment. Aerial mycelium was absent in very young cultures, but it appeared in about 2 weeks in the form of a white powdery crust. The organism proved to be gram-positive and acid-fast. A surface pellicle was produced in glucose broth. In dilute gelatin (0·4%) it produced globose colonies and gave a positive reaction with ninhydrin.[6] It hydrolysed casein, tyrosine, gelatin and starch but xanthine and hypoxanthine were not hydrolysed. It reduced nitrate to nitrite. It utilized citrate, pyruvate and succinate. Benzoate was not utilized. Acid was formed in glucose, galactose, inositol, mannitol, mannose and glycerol.

None of the 4 mice inoculated with 1 ml. of the suspension of *N. brasiliensis* survived more than 24 hours. From each of the remaining two groups of mice 2 animals were sacrificed after 1 week and the remaining two after 3 weeks. Lesions were found on the spleen, liver, kidney and peritoneum in all these animals. Histologically, these lesions were mostly in the form of circumscribed abscesses containing round,

oval and multilobulated granules (Fig. 2) which were gram-positive. The granules measured

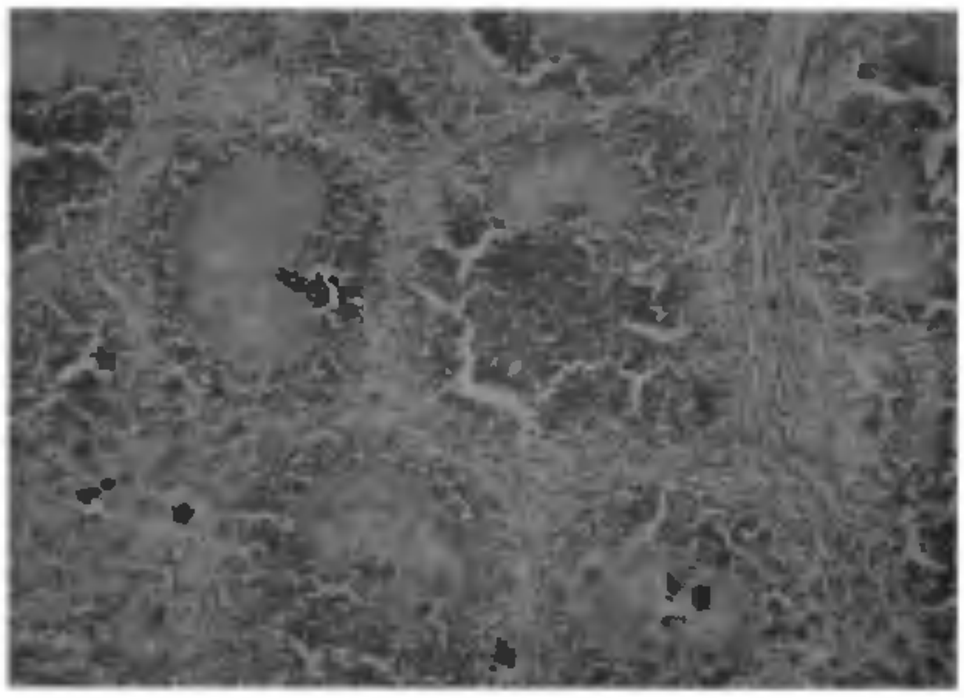

FIG. 2. Section through the liver of a mouse inoculated intraperitoneally with *N. brasiliensis*, showing abscesses containing granules. Hematoxylin and eosin stain, × 70.

50–500 μ in size. *N. brasiliensis* was recovered in culture from the lesions.

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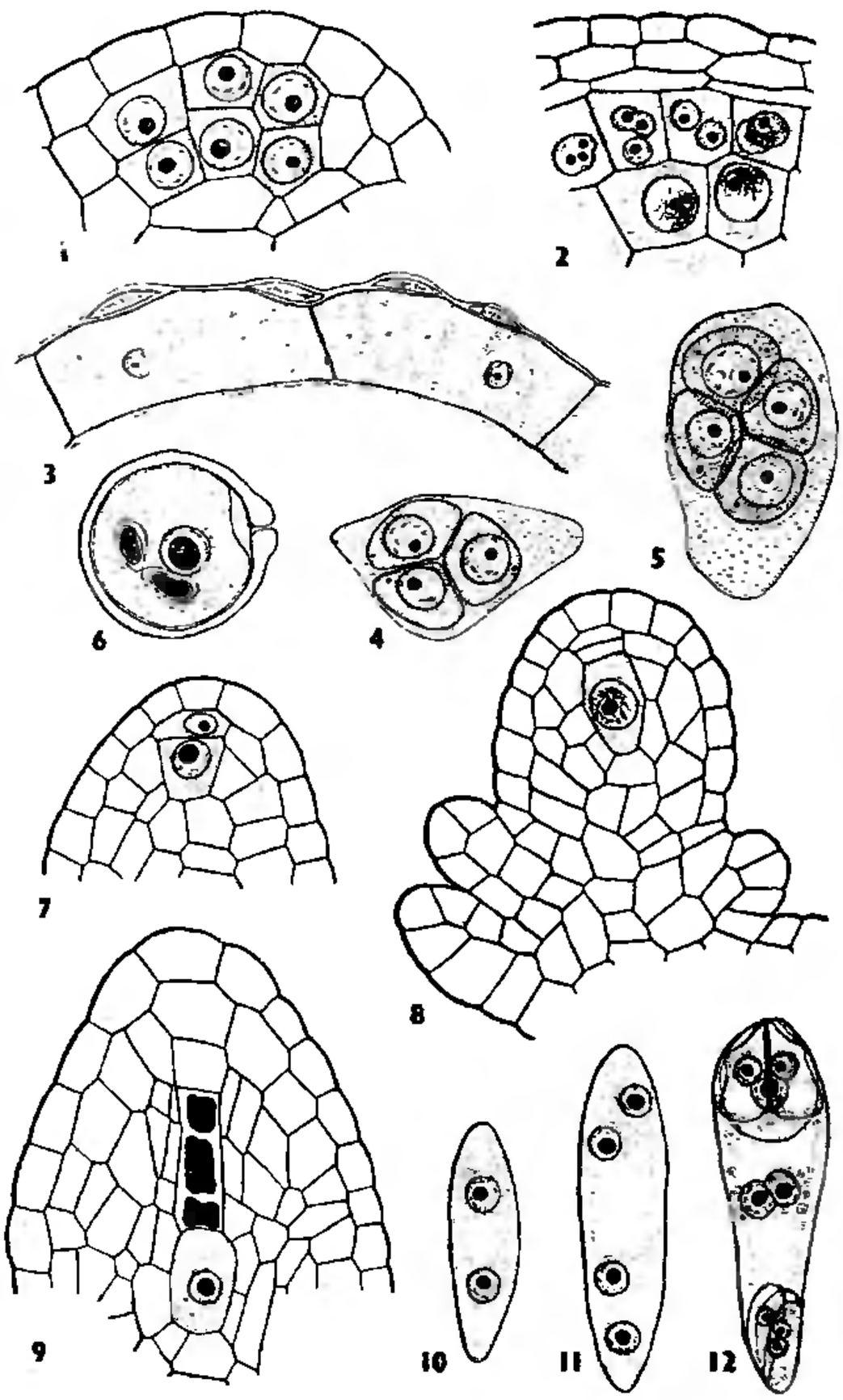
A NOTE ON THE SPOROGENESES AND THE GAMETOPHYTES OF *LAUREMBERGIA HIRSUTA* (W. et A.) SCHIND.

THE genus *Laurembergia* belongs to the family Haloragaceæ, tribe Halorageæ. The only embryological work on this genus is that of Bley.[1] Gamble[2] reported two species from South India, *viz.*, *L. brevipes* (W. et A) Schindler (= *Serpicula brevipes* W. et A) and *L. hirsuta* (W. et A) Schindler (= *Serpicula hirsuta* W. et A). The present note deals with the sporogeneses and the development of male and female gametophytes in *Laurembergia hirsuta*. A detailed paper on the embryology of this species will appear elsewhere.

Laurembergia hirsuta is a small, decumbent, branching herb. The flowers are axillary and arranged in dichasial clusters. They are monoecious, tetramerous, actinomorphic and epigynous. A group of 3-4 hypodermal archesporial cells is differentiated at each corner of the four-lobed young anther (Fig. 1). The latter



FIGS. 1–12. *Sporogenses and the male and female gametophytes.* Fig. 1. T.S. of portion of anther showing the primary parietal layer and the sporogenous layer, × 650. Fig. 2. T.S. of portion of anther showing the tapetum and the microspore mother cells, × 430. Fig. 3. Fibrous endothecium, × 430. Fig. 4. Tetrahedral tetrad, × 650. Fig. 5. Decussate tetrad, × 650. Fig. 6. Three-celled pollen grain, × 650. Fig. 7. Young ovule showing the primary parietal cell and the sporogenous cell, × 430. Fig. 8. Young ovule showing the megaspore mother cell and two parietal cells, × 430. Fig. 9. Linear tetrad showing the functional megaspore and three degenerating ones, × 430. Fig. 10. Two-nucleate embryo sac, × 1,455. Fig. 11. Four-nucleate embryo sac, × 430. Fig. 12. Eight-nucleate embryo sac, × 430.

occasionally becomes 3-lobed due to the fusion of two adjacent microsporangia. The archesporial cells divide periclinally forming a primary parietal layer and a primary sporogenous layer (Fig. 1). The former by further periclinal and anticlinal divisions forms an endothecium, a middle layer and a glandular tapetum (Fig. 2). As the anther matures the tapteum and the middle layer degenerate, while